

From: Ungar, Susan
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To: STIC-ILL
Subject: Papers for Examination of SN 09234290

Hi

I need the following papers to examine 09/234,290, this is a RUSH since this case is due this biweek.

1. Yoon et al, Annals of the NY Academy of Sciences, 2001, 928:200-211
2. Poulton et al, Diabetes/Metabolism Research and Reviews, 2001, 17(6)429-435 *****
3. Hanninen et al, Immunological Reviews, 2000, 173:109-119
4. Green et al (Immunological Reviews, 1999, 169:11-22
5. Simone et al, Diabetes Care, 1999, 22 Suppl 2 B7-B15
6. Palmer, J. Clin. Investigation, 2001, 108(1)31-33
7. Seddon et al (Biochem Soc. Transactions, 1997, 25(2)620-624)
8. Reddy et al, Histochemical Journal, 2000, 32(4)195-206
9. Ylinen et al, Pancrea, 2000, 20(2)197-205
10. Sainio et al, Pancrea, 1999, 18(3)282-293
11. Alamunits et al, Clinical and Experimental Immunology, 1999, 115(2)260-267.

I also need an entire volume, Cohen et al (Autoimmune Disease Models, A Guidebook, Academic Press, San Diego, 1994

Thanks
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703-305-2181
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Immunologic "Vaccination" for the Prevention of Autoimmune Diabetes (Type 1A)

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Diabetes type 1A is an autoimmune condition characterized by lymphocytic infiltration of islets and selective destruction of insulin-secreting β -cells. Numerous investigators have prevented diabetes in animal models with a variety of antigens and routes of administration. It is also now possible to identify high-risk individuals even before the appearance of autoantibodies. These advances have created the opportunity to design and begin human prevention trials. This review focuses on a variety of immunomodulatory approaches (including administration of adjuvants, autoantigens, T-cells, T-cell receptors, and DNA) that we have collectively termed immunologic "vaccination." In addition, we discuss the potential benefits and dangers of these approaches and issues relating to the design of human trials.

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Traditional vaccines consist of killed or attenuated organisms that prime the immune system and generate a protective immune response on subsequent exposure to the infectious agent. The important search for infectious agents linked to autoimmunity continues. Nevertheless, this association is far from clear for most forms of human autoimmune disease, including diabetes mellitus type 1A (1). As a result, a traditional vaccine to prevent most autoimmune diseases is not possible. In contrast, significant advances have been made in our understanding of the immunology of many autoimmune conditions. These advances have led to trials in animal models of immunologic "vaccines" that seek to modify the autoimmune response and prevent progression to disease. Although there is no ideal single term to describe the variety of techniques used to modify the autoimmune process, the

National Institute of Allergy and Infectious Diseases is sponsoring a conference on this topic and selected the term *vaccination* to describe different forms of autoantigen therapy to modify the autoimmune process. Other terms used by authors include *immunomodulation*, *immunotherapy*, and *tolerance induction*.

The hallmark of diabetes is lymphocytic infiltration of pancreatic islets containing insulin-secreting β -cells (2,3). Although T-cells appear to be a critical component of this process, autoantibodies to specific islet antigens can be detected in the serum of prediabetic individuals. Investigators have now characterized many of these autoantigens, including immunodominant epitopes of insulin (4) and GAD (5-7). Characterization of T-cell responses and T-cell receptors for specific antigens is an emerging field. These advances have led to the development of nontraditional "vaccines" that modify a

potentially autoreactive and destructive immune response and prevent or delay diabetes in animal models such as the NOD mouse. This information has now been used as the basis for large-scale human trials for the prevention of autoimmune diabetes in relatives of affected patients.

This review focuses on these immunomodulatory approaches to prevent autoimmune diabetes, with supporting information from other disease models, such as experimental autoimmune encephalomyelitis (EAE). The different areas to be reviewed include 1) vaccination with "nonspecific" adjuvants; 2) autoantigen administration by different routes (subcutaneous, oral, intravenous, nasal, inhaled); 3) whole T-cell vaccination; 4) T-cell receptor (TCR) vaccination; 5) DNA vaccination; 6) potential benefits and dangers of autoimmunity vaccines; and 7) issues relevant to the design of "vaccine" trials for type 1 diabetes.

"ADJUVANT" VACCINATION —

Adjuvants, such as complete Freund's adjuvant, are usually administered to enhance an immune response. Thus, it was of considerable interest that a single injection of Freund's adjuvant prevented the development of diabetes in NOD mice (8,9). Administration of complete (containing mycobacteria) but not incomplete Freund's adjuvant protected from diabetes. Despite the dramatic prevention of diabetes, administration of adjuvant did not prevent development of insulinitis. A shift in cytokine production of infiltrating lymphocytes correlates with protection (10-13).

After the discovery of the effectiveness of Freund's adjuvant, bacillus Calmette-Guerin (BCG) vaccination was also found to prevent diabetes in NOD mice (14). It is of interest that heat shock proteins are a component of both Freund's adjuvant and BCG and have been a focus of investigation in NOD mice (15). Success of BCG vaccination in animal studies has prompted trials in humans. A small, nonrandomized trial of BCG administration in new-onset diabetic patients was carried out in Israel, and BCG was reported to enhance remissions of overt diabetes (16). Unfortunately,

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Abbreviations: BCG, bacillus Calmette-Guerin; DPT-1, Diabetes Prevention Trial; EAE, experimental autoimmune encephalomyelitis; ICA, islet cell autoantibody; IL, interleukin; PCR, polymerase chain reaction; TCR, T-cell receptor; TGF, transforming growth factor; V, variable.

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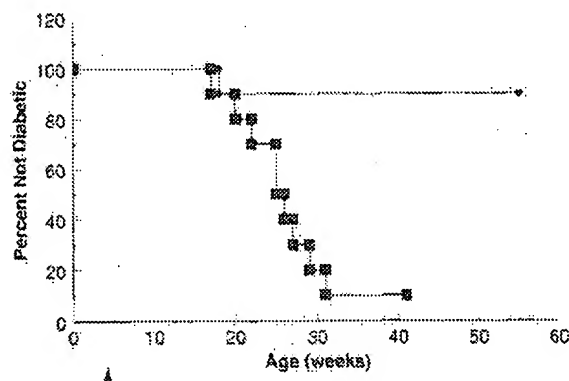


Figure 1—Prevention of progression to diabetes by administration of insulin B-chain peptide (B:9-23). Female NOD mice were given a single subcutaneous injection of insulin B:9-23 peptide in incomplete Freund's adjuvant at 4 weeks of age. Controls received tetanus toxin 830-840 in incomplete Freund's adjuvant. From Daniel and Wiegmann (27). ■, control; ●, B:9-23.

this study was not a randomized placebo-controlled trial with a biochemical endpoint such as C-peptide secretion. Klingensmith et al. (17) at the Barbara Davis Center have now analyzed a randomized placebo-controlled trial of BCG vaccination and found no protection. A comparison of nicotinamide or a combination of nicotinamide and BCG also failed to demonstrate a benefit of BCG in newly diagnosed patients (18).

AUTOANTIGENS AS IMMUNOLOGIC VACCINES

Multiple autoantigens have been implicated in animal and/or human autoimmune diabetes. These include insulin (19) and proinsulin, GAD65 and GAD67 (20,21), membrane granule proteins with homology to tyrosine phosphatases (termed ICA512 or IA-2), a related molecule (termed phogrin, ICA512, or IA2B), a 38-kD autoantigen (22), and less well-characterized autoantigens including an islet neuroendocrine ganglioside (23-26). Autoantigen characterization usually followed the detection of autoantibodies to these molecules. Although an immune response to these autoantigens is a natural part of disease development, several of these molecules, when administered appropriately, can also prevent or delay onset of diabetes.

SUBCUTANEOUS AUTOANTIGENS

Multiple reports document the efficacy of subcutaneous administration of islet autoantigens to delay or prevent diabetes in animal models. Both insulin and GAD have been extensively

studied, including characterization of specific peptides that confer dominant immunoprotection (6,27-32). Indeed, a single subcutaneous injection of the insulin B-chain peptide B:9-23 prevents 90% of diabetes in NOD mice (Fig. 1) (27,28). The importance of the insulin B-chain was discovered by an additional study where multiple injections of insulin and the insulin B-chain, but not the A-chain, protected NOD mice from diabetes (33). These experiments were performed starting at 4 weeks of age, at a time when islet-infiltrating T-cells are first apparent. Daily insulin therapy preceding adoptive transfer of diabetes in the NOD/acid also resulted in a significant delay but did not reduce the final incidence of diabetes (34).

The mechanism by which subcutaneous insulin prevents diabetes is currently unknown. One hypothesis is that the therapy induces energy of potentially autoreactive T-cells and results in tolerance to the specific autoantigen. Although this is certainly possible, the hypothesis does not explain how a single autoantigen such as GAD influences the other autoimmune T-cells directed at autoantigens such as insulin. An alternate hypothesis is that the therapy induces regulatory T-cells, which then localize in the islets and downregulate the autoimmune response to all antigens. Indeed, Zekzer et al. (35) isolated a CD4⁺ T-cell clone from NOD mice that prevents the development of diabetes upon transfer into prediabetic mice (Fig. 2). This clone secretes transforming growth factor β (TGF β) and, of note, was found to react with insulin peptide B:9-23. It is likely that recognition of peptide B:9-23 targets T-cells to islets, and the lymphokines produced determine whether a given T-cell clone is pathogenic or protective.

ORAL THERAPY—Oral feeding of autoantigens, although not a typical "vaccine" as the intervention must be continued for long periods, results in immunomodulation and decreased disease in different models of autoimmunity (36-39). Five-week-old female NOD mice were fed porcine insulin (1 mg) twice a week for 5 weeks and then once a week for up to a year (37). This treatment both delayed the onset and reduced the incidence of diabetes. Spleen cells from treated animals also reduced disease when transferred with

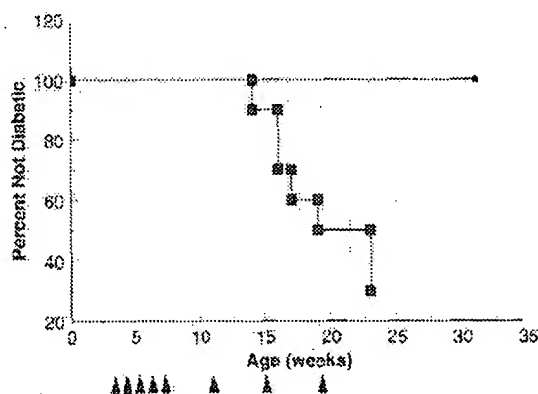


Figure 2—Prevention of diabetes by administration of a protective NOD T-cell clone. Female NOD mice were injected weekly for 4 weeks and then every 4 weeks with 10^6 T-cells. Clone 2H6 was isolated from peripancreatic lymph nodes of NOD mice and reacts with insulin B-chain peptides, including the B:9-23 sequence. Controls were injected with saline. From Zekzer et al. (35). ■, control; ●, clone 2H6.

diabetic NOD spleen cells, suggesting the presence of regulatory cells in the insulin-fed animals. Oral GAD (500 µg twice weekly) was fed to NOD mice in a similar protocol and resulted in delayed diabetes onset (28). Spleen cells from these GAD-treated mice, however, were unable to prevent diabetes in the adult transfer model.

An additional elegant model where oral insulin appears to protect from diabetes is a transgenic mouse where the nucleoprotein of the lymphocytic choriomeningitis virus is under control of the rat insulin promoter (40). Normally diabetes develops in >95% of animals after lymphocytic choriomeningitis viral infection in a CD8⁺ cytotoxic dependent manner. Twice-weekly oral treatment with 1 mg of insulin for 2 months resulted in protection of >50% of the animals. Islets of the protected mice still had insulinitis, but a majority of the lymphocytes produced interleukin (IL)-4, IL-10, and TGF-β rather than predominantly γ-interferon, as seen in the diabetic transgenic mice. This finding supports a hypothesis that protection from regulatory T-cells is induced following oral insulin.

Several studies suggest that the dose of the oral antigen is critical in the mechanism of the response. This was first demonstrated in the EAE model, where low doses of oral myelin basic protein resulted in transferable suppression associated with an increased secretion of TGF-β and IL-4, whereas high doses led to anergy and an inability to transfer protection (41). Similar studies with oral insulin in the NOD mouse confirmed the importance of antigen dose: animals receiving 1 mg were protected, whereas those receiving the higher dose of 5 mg had an acceleration of disease (37). Similar studies have documented the presence of T-cells with a TH2-like pattern of cytokines (IL-4, IL-10, TGF-β) in peri-islet infiltrates of NOD mice fed insulin (42).

Most reports of oral tolerance suggest a need for antigen administration for an extended time period. The exception has been a report of orally administered insulin linked to the cholera toxin B subunit, which protected NOD mice from diabetes after a single dose (43). Such approaches may hold potential for development of an oral diabetes vaccine.

Initial human trials of orally administered antigen in patients with multiple sclerosis and rheumatoid arthritis suggested a possible benefit (44,45). Unfortunately, a double-blind, placebo-controlled, phase III multicenter trial of oral myelin in 515

relapsing-remitting multiple sclerosis patients failed to show a benefit. Phase II clinical trials using type II collagen in rheumatoid arthritis and S-antigen in uveitis are in progress. Individuals identified at moderate risk for type 1 diabetes are now being randomized for oral insulin or placebo as part of the Diabetes Prevention Trial (DPT-1).

INTRAVENOUS SOLUBLE AUTOANTIGEN

— Intravenous injection of soluble insulin was initially shown to prevent subsequent immune responses to insulin in mouse strains not susceptible to type 1 diabetes (46,47). Bovine and ovine insulin, when injected into NOD mice at 4 weeks of age, were effective at reducing but not preventing insulinitis and diabetes (48). This study also demonstrated reduced insulin autoantibodies in the insulin-treated mice. Intravenous injection of soluble GAD into 3-week-old female NOD mice also reduced insulinitis and diabetes development and decreased T-cell proliferation responses to GAD (49).

The mechanism of protection of parental soluble antigen, however, remains unclear. One hypothesis is that intravenous or subcutaneous insulin influences autoimmunity by decreasing β-cell synthesis and secretion of autoantigens (β-cell rest). This hypothesis was first proposed in the BB rat model, where hypoglycemic insulin doses produced a protective effect (50). Diazoxide, with inhibition of endogenous insulin and resulting hyperglycemia, also offered protection (51). In subsequent NOD studies, however, insulin peptides or forms of metabolically inactive insulin provided a degree of protection similar to that of intact insulin (27,52). These results suggest that immunomodulation, rather than β-cell rest, is an equal if not dominant mechanism of protection in the NOD.

NASAL AND INHALED AUTOANTIGENS

— The immunodominant insulin B:9-23 peptide can also be given to NOD mice by an intranasal route. This method of administration, when given monthly starting at 4 weeks of age, significantly delays the onset of diabetes and reduces the incidence of diabetes, although not as effectively as the single dose of subcutaneous peptide in incomplete Freund's adjuvant (27). The mechanism of nasal protection has not been as extensively studied as oral tolerance, although a similar process is postulated. Insulin B:9-23 reac-

tive, CD4⁺ T-cells with a Th2 phenotype can be isolated from the draining lymph nodes of B:9-23 nasally treated animals. Surprisingly, these Th2 cells accelerate the development of diabetes in young NOD mice (53), suggesting that the Th1 (pathogenic) versus Th2 (protective) paradigm may be an oversimplification.

Aerosolized insulin also appears to reduce the incidence of diabetes in NOD mice treated for 10 consecutive days and then weekly starting from 4 weeks of age (54). The treated mice had increased insulin autoantibodies, decreased splenocyte proliferation to B:9-23 peptide and GAD, and increased IL-4 and IL-10 secretion. A similar reduction was also seen in animals who started treatment at 7 weeks of age, suggesting that initiation of therapy after the onset of autoimmunity can be effective. A small population of CD8⁺ γδ T-cells appeared best able to suppress the adoptive transfer of diabetes in this system.

WHOLE T-CELL VACCINATION

Numerous experimental and spontaneous autoimmune conditions are now known to be T-cell mediated. In the process of studying these conditions, antigen-specific T-cell lines and clones have been isolated and found to transfer disease. When these T-cells are administered under certain conditions, however, a protective effect is observed. In general, the T-cell line or clone must first be activated and then attenuated, with irradiation for example, to prevent disease. This approach has successfully prevented disease in several different murine experimental autoimmune models, including EAE, (55) uveoretinitis (56), thyroiditis (57), and collagen-induced arthritis (58).

Whole T-cell vaccination has also been used to treat the spontaneous autoimmune diabetes of the NOD mouse. A vaccination with T-cells recognizing a peptide of the 65-kDa heat shock protein significantly reduced the incidence of diabetes (59). Others have vaccinated NOD mice with lymphocytes from spleens of diabetic mice, which delayed the onset and possibly reduced the incidence of diabetes (60). A second group used an anti-TCR Vβ 8 monoclonal antibody to isolate and activate a subset of T-cells from diabetic spleens (61). These cells were able to suppress diabetes in an adoptive transfer model and completely prevent both insulinitis and diabetes in young NOD female mice.

The mechanism of T-cell vaccination was initially postulated to be due to a spe-

cific anti-idiotypic T-cell response directed at the TCR of the clone used for vaccination. Examples of these "anti-TCR" T-cells have been isolated from animals vaccinated with myelin basic protein specific T-cells from the EAE model (62). T-cell vaccination also results in a nonspecific, delayed type hypersensitivity response directed at shared antigens on activated T-cells (63). Recently, another group reported that T-cell vaccination for EAE also induces a humoral anti-T-cell response capable of inhibiting T-cell proliferation and improving disease (64). This autoantibody response was not idiosyncratic, however, as vaccination with an unrelated T-cell clone produced sera equally effective in suppressing EAE.

TCR PEPTIDE VACCINES— TCR peptide vaccines have been developed for a variety of autoimmune conditions associated with autoreactive T-cells containing a single predominant TCR chain. These conditions include EAE, experimental allergic neuritis (65), experimental allergic uveitis (66), and collagen-induced arthritis (67). Investigators have attempted to immunize animals with peptides from the hypervariable regions (CDR1, CDR2, or CDR3) of the TCR variable (V) chain. This approach has the potential advantage of generating an immune response to only the autoreactive subset of T-cells.

Much of the initial work focused on the EAE model, with reports of prevention or reduction of the severity of symptoms in animals vaccinated with peptides from either the CDR2 or CDR3 regions of TCR β -chains (68,69). TCR V β -chain peptide immunization also prevented the induction of collagen-induced arthritis in mice (70,71). Attempts to repeat some of this work, however, have produced variable results, including one report where the peptide vaccination increased the severity and course of disease (72).

Different mechanisms have been suggested for the action of TCR peptide vaccines. Early reports of protection in the EAE model were not accompanied by evidence for direct T-T cell interactions. Subsequently, increasing evidence has accumulated for the existence of T-cells that recognize specific TCR peptides (73). Immunogenic TCR peptides induced CD4⁺ T-cells, which recognized both recombinant TCR and the original pathogenic T-cell (74). Another group has further defined a subgroup of CD4⁺ regulatory T-cells specific for an immunodominant V β 8.2 peptide in both

EAE and collagen-induced arthritis (71,75). These cells appear to be involved in both the protection of animals after V β 8.2 peptide immunization and during spontaneous recovery from EAE. In addition, deletion of these regulatory T-cells with a specific monoclonal antibody increased the severity and duration of EAE. Cytotoxic (CD8⁺) peptide-specific T-cells have also been described after immunization with V β 8.2 epitopes (76). In this case, there was no evidence of deletion of V β 8.2⁺ cells but rather a suppression of stimulation with a V β 8.2-specific monoclonal antibody. In contrast, V β 10 peptide immunization for collagen-induced arthritis resulted in the absence of V β 10 cells in lymph nodes of protected mice compared with untreated mice, raising the possibility of deletion rather than anergy of the target cell population (70).

The success in treating animal models has prompted the investigation of treating human autoimmune conditions with TCR peptide vaccines. A V β 17 TCR peptide vaccine has been investigated in a phase I trial for rheumatoid arthritis (77). Although this was an uncontrolled trial, decreases in patient joint scores were observed after vaccination. In addition, activated V β 17 T-cells were decreased in the peripheral blood of a majority of patients given higher doses of the vaccine, and T-cell proliferation to the V β 17 peptide was detected in 40% of immunized patients.

Until recently, there has been less interest in the possibility of TCR peptide vaccines for diabetes. In contrast to the reported V β restriction of many experimental autoimmune models and some human diseases, most investigators have not found a similar pattern in the NOD mouse. Anchored polymerase chain reaction (PCR) analysis of NOD thymus or spleen cells did not reveal any V β bias (78). The V β repertoire in infiltrated islets was also diverse (79). Several investigators have sequenced both the V β and V α TCR chains from small panels of NOD, CD4⁺ T-cell clones derived from either the spleen or lymph nodes (four clones) (80) or from islets (five clones) (81). Both groups reported heterogeneous V β and V α utilization. Although both panels of clones were reactive with islets, the antigens recognized by the clones were not known. Other experiments argue against a role for V β restriction in NOD diabetes. Selective breeding to delete V β subsets did not prevent diabetes (82,83). Furthermore, transgenic introduction of a V β 8.2 TCR chain

into the NOD (with allelic exclusion of >98% of endogenous V β chains) did not alter the disease course (84).

An alternate approach, emphasized by our group at the Barbara Davis Center, focused on the isolation and characterization of panels of T-cells with reactivity to defined autoantigens such as insulin and GAD. Wegmann and colleagues (85) isolated large numbers of T-cells from the islets of NOD mice, which were subsequently discovered to have reactivity with the insulin B-chain peptide consisting of amino acids 9–23 (B:9–23). Unlike the T-cells in the EAE model, these anti-insulin cells contained heterogeneous TCR β -chains. The TCR α -chains, however, were dramatically restricted with the predominant use of a V α 13.3 segment combined with either J α 45 or J α 34. This restricted TCR α -chain predominance raises the possibility of developing a TCR α -chain peptide vaccine for autoimmune diabetes in NOD mice. Success of a vaccine targeting only insulin-reactive T-cells would further contribute to testing the hypothesis that insulin is a critical if not dominant autoantigen for diabetes. Ongoing studies are examining the TCRs of GAD peptide reactive clones.

More limited data are available on diabetes-associated TCRs in human disease. One report described a predominance of V β 7 in CD4⁺ T-cells derived from islets of two new-onset diabetic patients (86). This result was not confirmed by a reverse transcription-PCR analysis on pancreas biopsy specimens from Japanese new-onset type 1 diabetic patients. They did, however, report significantly fewer V α transcripts in type 1 patients versus control subjects, with V α 4 and V α 6 occurring most commonly (87). Insulin B:9–23 reactive T-cell clones have recently been isolated from patients with type 1 diabetes (P. Gottlieb, personal communication), and TCR analysis is in progress by our group. The presence of human TCR restriction for a dominant autoantigen, such as insulin, would create an opportunity to develop a TCR peptide vaccine for diabetes in humans.

DNA VACCINATION— Several groups are currently investigating the possibility of administering naked DNA as a form of vaccination to modify autoimmunity. The approach of DNA vaccination was an accidental discovery during attempts to insert DNA into muscle cells. The proposed mechanism is that the injected DNA is taken up by cells, where it is transcribed

into mRNA and expressed as protein. Peptides from the protein can then be presented in the context of both class I and class II major histocompatibility complex molecules, which results in both cellular and humoral immune responses (88). Although the protein can be documented in injected muscle cells, an immune response is likely mediated by specialized antigen-presenting cells, such as dendritic cells.

Although most studies have investigated DNA vaccination to treat infectious disease, two reports describe the use of DNA to modulate autoimmunity. In one case, investigators injected DNA encoding the mycobacterial heat shock protein 65 into the muscle of Lewis rats susceptible to adjuvant-induced arthritis (89). They then documented the expression of hp65 within muscle cells, increased specific humoral and cellular immune responses, and a decrease in the severity of arthritis that generally correlated with hp65 antibody titers. A second group immunized EAE-susceptible mice with DNA encoding the TCR variable region gene V β 8.2 (90). This intramuscular vaccination also resulted in both cellular and humoral immune responses to the V β 8.2 TCR. In addition, immunized mice were protected from subsequent EAE induction. Data suggested that this protection was not due to a deletion of V β 8.2 cells but rather to a shift in cytokine profiles of the target cells from a Th1 to a Th2 phenotype. Such a response would be potentially beneficial for other autoimmune conditions, such as diabetes, that also appear to be mediated by T-cells with a Th1-like cytokine profile.

POTENTIAL BENEFITS AND DANGERS OF VACCINES FOR AUTOIMMUNE DISEASE

As with diabetes, most autoimmune diseases do not have excellent treatments. Insulin replacement, for instance, is an imperfect art requiring significant lifestyle changes for the individual. The resulting glucose control is associated with both short-term complications, such as hypoglycemia, and long-term complications, such as microvascular disease (retinopathy, nephropathy, and neuropathy) and macrovascular disease (heart disease and stroke). The personal and societal costs of treating chronic conditions of this type are tremendous. As a result, the development of a vaccine to modulate the immune process, even if it meant only delaying the diagnosis of the disease several years, would be worthwhile.

The ability to prevent the disease entirely would be a tremendous accomplishment.

An obvious potential danger of immunologic "vaccines" for autoimmunity is the possibility of stimulating the immune system and worsening the process or even initiating autoimmunity. Approaches utilizing autoantigens or DNA constructs for autoantigens would be most likely to produce this result, as pathogenic autoimmune T-cells target these same autoantigens. Neonatal injection of an ovarian peptide, for example, results in autoimmune ovarian disease and autoantibodies to the peptide in female mice (91). Indeed, an early model of autoimmune diabetes in rabbits was induced by the injection of insulin preparations in complete Freund's adjuvant (92). In contrast, numerous attempts to induce diabetes with defined autoantigens in different strains of mice and rats have been unsuccessful. Furthermore, there have been no reports of progression to autoimmune diabetes in nondiabetic patients treated with intravenous or subcutaneous insulin (e.g., insulin shock therapy) (93), and preliminary evidence suggests that the administration of subcutaneous and intravenous insulin delays the development of diabetes in nondiabetic anti-islet autoantibody positive relatives (94).

Two recent articles highlighted the potential for immunologic vaccination to accentuate autoimmunity. In one model, a transgene was utilized to direct islet synthesis of ovalbumin, and T-cells were infused with a monoclonal TCR that recognizes ovalbumin (95,96). The feeding of large amounts of ovalbumin increased the development of diabetes in this model, though in the absence of T-cell infusion, diabetes did not develop. In a primate (marmoset) model of EAE, intraperitoneal administration of myelin oligodendrocyte glycoprotein led to severe central nervous system lesions after initial transient improvements (97). These two model systems argue for caution in the testing of immunologic vaccines.

T-cell vaccines have the potential of generating immune responses to multiple T-cell molecules. This is not necessarily negative, and it may even contribute to immunoregulation. Nevertheless, the potential danger of establishing an autoimmune response to T-cells must be considered. TCR peptide vaccines avoid this potential problem by targeting only a subset of T-cells containing the target receptor element. Unexpected problems may also

arise, however, once some of these vaccines progress to clinical trials. A trial of V β peptides in patients with multiple sclerosis produced one patient who developed a skin vasculitis after receiving the highest dose of peptide (98). The vasculitis resolved with a short course of prednisone and discontinuation of the peptide.

DNA vaccination could potentially induce anti-DNA autoimmunity. Indeed, BALB/c mice immunized with DNA had measurable levels of anti-DNA autoantibody titers but no evidence of glomerulonephritis or other autoimmune disease (99). Because of this result, the authors also investigated the production of anti-DNA antibodies in lupus-prone (NZB \times NZW) F1 mice and found that vaccination did not alter the onset or course of lupus in these animals. Others have been concerned about the potential of DNA vaccines to integrate into genomic DNA, disrupting critical genes that could contribute to tumor formation. The estimated risk, however, is very low, and National Institutes of Health trials have already been approved for healthy volunteers to test DNA vaccines directed at an HIV envelope protein and the influenza nucleoprotein. Such theoretical risks should be acceptable to individuals at risk for autoimmune diseases, such as diabetes, that are associated with significant morbidity and mortality.

CURRENT "ANTIGEN"-BASED TRIALS OF DIABETES PREVENTION

The largest antigen based trial for the prevention of type 1 diabetes is the DPT-1, for which >58,000 first-degree relatives of patients with type 1 diabetes have been screened for expression of cytoplasmic islet cell autoantibodies (ICAs). ICA-positive relatives are "staged" with determination of insulin autoantibodies, first-phase insulin secretion upon intravenous glucose tolerance testing, and HLA typing. The presence of the protective HLA haplotype DQA1*0102, DQB1*0602 is an exclusion criterion. High-risk relatives with low first-phase insulin secretion are randomized to intravenous and low-dose subcutaneous insulin therapy versus close observation, whereas relatives with normal first-phase insulin secretion but expressing anti-insulin autoantibodies are randomized to receive either oral insulin or oral placebo. The trial has been well accepted. A total of 219 relatives have been randomized to the parenteral arm and 130 to the oral arm as of March 1998. The study is based

in part on a small pilot trial of combination intravenous and subcutaneous insulin, which is continuing (94). To date, eight of eight nontreated relatives became diabetic within 3 years, whereas two of nine insulin-treated patients remain nondiabetic, with the longest follow-up at 9 years. Two other small pilot trials, one performed in Germany (100) and the other in Israel, suggest a similar delay in onset to diabetes in very high-risk individuals.

DESIGN OF "VACCINATION" TRIALS FOR PREVENTION OF DIABETES OR AUTOIMMUNITY

The development of quantitative autoantibody assays has contributed to the collection of significant "natural" history and pilot trial data. This information suggests that one could now design larger trials to intervene at several possible stages in the autoimmune process. To understand the benefits and disadvantages of these different trials, one must distinguish between individuals at genetic risk (based currently on HLA genes and insulin region polymorphisms) but with no evidence yet of autoimmunity (antibodies to islet proteins such as insulin, GAD, or ICA512), individuals with signs of autoimmunity but with normal insulin secretion, individuals with impaired insulin secretion (based on first-phase insulin release), or finally those with a new diagnosis of diabetes who still produce small amounts of insulin as measured by C-peptide. These individuals represent a spectrum of autoimmunity where those with only the high-risk genes have 100% of their original β -cell mass, whereas individuals at diabetes diagnosis generally have <10% of functioning β -cells. Other individuals, those who carry the protective HLA genes DQA1*0102 and DQB1*0602, may develop evidence of autoimmunity (such as a single autoantibody) but rarely progress to diabetes.

A trial to prevent the appearance of autoantibodies, for example, would have to focus on a very high-risk group. The Diabetes Autoimmunity Study in the Young (DAISY) from Denver suggests that 40% of first-degree relatives of patients with type 1 diabetes who have the highest-risk HLA genotype DR3/4 (DQB1*0302) will develop persistent expression of biochemically determined autoantibodies by 2 years of age (101,102). A preliminary analysis suggests that 70% of these individuals will develop diabetes within 3 years. Studies from Ger-

many of offspring of parents with type 1 diabetes find a similar risk of conversion to autoantibody positivity (19). A power calculation estimates a need for 182 individuals randomized to either therapy or placebo "immunization" to detect a 50% decrease in events. Assuming a 50% participation rate, one would need to screen 3,640 newborn first-degree relatives to identify the 364 (10%) high-risk individuals eligible for enrollment. Other data suggests that a majority of the individuals positive for one antibody will eventually develop two or more antibodies prior to diagnosis. As a result, one might also consider a trial to prevent subsequent antibody development in a group positive initially for only a single antibody. Such an approach might limit the number of individuals needed for the study while still allowing an intervention early enough in the autoimmune process to improve chances for a positive result.

CONCLUSIONS — Although many different therapies will prevent or delay diabetes in the NOD mouse, few will likely have the same impact on humans. Small pilot trials suggest that human autoimmune diseases may be less likely to respond to interventions than animal models. Unfortunately, most of the human trials tested interventions at a very late stage of the autoimmune process, such as at diagnosis of new-onset diabetes. It seems reasonable that a therapy unsuccessful at this stage could have a very different result if initiated before the appearance of autoantibodies. Although insulin peptide B:9-23 decreases the development of diabetes in NOD mice even late in the disease process (12- and 16-week NOD mice; P. Gottlieb, personal communication), the treatment is most effective if administered before detectable autoimmunity (+ weeks of age). Although this suggests that it may be possible to test immunologic therapies late in the disease process, similar to the design of the current DPT-1 trial, administration of a "vaccine" before the appearance of autoantibodies may be more likely to produce positive results. As a result, one must be careful not to eliminate potentially useful treatments based only on the results of late-stage autoimmunity trials. Hopefully, early-stage intervention trials will be conducted in the near future.

Many of the major decisions concerning trials of immunologic "vaccination" to prevent diabetes have already been addressed in current preventive trials and studies of the

natural history of type 1 diabetes. We are rapidly approaching the point where it would be possible to design and implement a trial to treat individuals "before" the development of autoantibodies. With HLA typing and family history of type 1 diabetes, one can identify individuals with a 40% risk of expressing autoantibodies. Improvements in genetic characterization and our understanding of early pathogenic events will likely increase predictability of activation of autoimmunity. Nevertheless, it is unlikely that 100% predictability will ever be achieved for any group lacking autoantibodies. As with any screening program, one must consider the risk of false positives and stigmatization. Until an effective treatment is available, genetic and antibody screening for diabetes should be performed for research purposes only.

Critical factors in the design of future intervention trials will include the dose of antigens and adjuvants and the unknown safety of the proposed regimens. These issues will likely be addressed through the following series of seven stages: Stage 1, testing different vaccines, adjuvants, doses, and routes of immunization in animal models; Stage 2, phase I administration to normal individuals to assess development of anti-islet autoantibodies or unexpected toxicity; Stage 3, evaluation in patients with new-onset type 1 diabetes, with the major outcome variable being loss of C-peptide secretion; Stage 4, trials in relatives of patients with type 1 diabetes who express anti-islet autoantibodies; Stage 5, trials in the highest genetic risk individuals—at present DR3/4 (DQB1*0302) relatives of patients with type 1 diabetes—who do not yet express anti-islet autoantibodies; Stage 6, trials in groups with a moderate genetic risk of type 1 diabetes; and Stage 7, true large-scale vaccination trials in the general population.

Though a pathway to the testing of immunologic "vaccination" for prevention of type 1 diabetes is relatively clear, it is likely that the actual path to this goal will be altered as our knowledge increases. Prior human trials utilizing incomplete Freund's adjuvant, and our own experience with insulin as an autoantigen in both the NOD mouse and humans, have contributed to this combination as a leading therapy for further evaluation at the Barbara Davis Center. Other centers will likely choose different approaches to move toward the ultimate goals of developing an immunologic "vaccine" to prevent diabetes

in susceptible individuals and halting recurrent autoimmunity after islet or pancreas transplantation.

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References

1. Rewers M, Norris JM: Epidemiology of type 1 diabetes. In *Type 1 Diabetes: Molecular, Cellular, and Clinical Immunology*. 1st ed. Eisenbarth GS, Lafferty KJ, Eds. New York, Oxford University Press, 1996, p. 172-208
2. Foulis AK, Liddle CN, Farquharson MA, Richmond JA, Weir RS: The histopathology of the pancreas in type 1 (insulin-dependent) diabetes mellitus: a 25-year review of deaths in patients under 20 years of age in the United Kingdom. *Diabetologia* 29:267-274, 1986
3. Bach JF: Insulin-dependent diabetes mellitus as an autoimmune disease. *Endocr Rev* 15:516-542, 1994
4. Daniel D, Gill RG, Schloot N, Wegmann D: Epitope specificity, cytokine production profile and diabetogenic activity of insulin-specific T cell clones isolated from NOD mice. *Eur J Immunol* 25:1056-1062, 1995
5. Tisch R, Yang XD, Liblau RS, McDevitt HO: Administering glutamic acid decarboxylase to NOD mice prevents diabetes. *J Autoimmun* 7:845-850, 1994
6. Tian J, Clare-Salzler M, Herschenfeld A, Middleton B, Newman D, Mueller R, Arita S, Evans C, Atkinson MA, Mullen Y, Sarvetnick N, Tobin AJ, Lehmann PV, Kaufman DL: Modulating autoimmune responses to GAD inhibits disease progression and prolongs islet graft survival in diabetes-prone mice. *Nat Med* 2:1348-1353, 1996
7. Endl J, Otto H, Jung G, Dreisbusch B, Donie F, Stahl P, Elbracht R, Schmitz G, Meinel F, Hummel M, Ziegler AG, Wank R, Schendel DJ: Identification of naturally processed T cell epitopes from glutamic acid decarboxylase presented in the context of HLA-DR alleles by T lymphocytes of recent onset IDDM patients. *J Clin Invest* 99:2405-2415, 1997
8. Sadelain MW, Qin HY, Lauzon J, Singh B: Prevention of type 1 diabetes in NOD mice by adjuvant immunotherapy. *Diabetes* 39:583-589, 1990
9. Sedelain MW, Qin HY, Sumoski W, Parfrey N, Singh B, Rabinovitch A: Prevention of diabetes in the BB rat by early immunotherapy using Freund's adjuvant. *J Autoimmun* 3:671-680, 1990
10. Rabinovitch A, Suarez-Pinzon WL, Lapchak PH, Meager A, Power RF: Tumor necrosis factor mediates the protective effect of Freund's adjuvant against autoimmune diabetes in BB rats. *J Autoimmun* 8:357-366, 1995
11. Shehadeh NN, LaRosa F, Lafferty KJ: Altered cytokine activity in adjuvant inhibition of autoimmune diabetes. *J Autoimmun* 6:291-300, 1993
12. Wang T, Singh B, Warrock GL, Rajotte RV: Prevention of recurrence of IDDM in islet-transplanted diabetic NOD mice by adjuvant immunotherapy. *Diabetes* 41:114-117, 1992
13. McInerney MF, Pek SB, Thomas DW: Prevention of insulinitis and diabetes onset by treatment with complete Freund's adjuvant in NOD mice. *Diabetes* 40:715-725, 1991
14. Harada M, Kishimoto Y, Makino S: Prevention of overt diabetes and insulinitis in NOD mice by a single BCG vaccination. *Diabetes* 39:85-90, 1990
15. Elias D, Markovits D, Reshef T, Zee R, Cohen IR: Induction and therapy of autoimmune diabetes in the non-obese diabetic (NOD/Lt) mouse by a 65-kDa heat shock protein. *Proc Natl Acad Sci USA* 87:1576-1580, 1990
16. Shehadeh N, Calcinaro F, Bradley BJ, Bruchlim I, Vardi P, Lafferty KJ: Effect of adjuvant therapy on development of diabetes in mouse and man. *Lancet* 343:706-707, 1994
17. Klingensmith G, Allen H, Hayward A, Stohry L, Humber C, Jensen P, Simoes E, Chase HP: Vaccination with BCG at diagnosis does not alter the course of IDDM (Abstract). *Diabetes* 46 (Suppl. 1):193A, 1997
18. Pozzilli P: BCG vaccine in insulin-dependent diabetes mellitus: IMDiAB Group. *Lancet* 349:1520-1521, 1997
19. Roll U, Christie MR, Fuchtnbusch M, Payton MA, Hawkes CJ, Ziegler AG: Perinatal autoimmunity in offspring of diabetic parents: the German Multi-Center BABY-DIAB study: detection of humoral immune responses to islet antigens in early childhood. *Diabetes* 45:967-973, 1996
20. Christie MR: Humoral immune markers: antibodies to glutamic acid decarboxylase. In *Prediction, Prevention, and Genetic Counseling in IDDM*. Palmer JP, Ed. Chichester, U.K., Wiley, 1996, p. 77-96
21. Falorni A, Ackelors M, Carlberg C, Daniels T, Persson B, Robertson J, Lernmark A: Diagnostic sensitivity of immunodominant epitopes of glutamic acid decarboxylase (GAD65) autoantibodies in childhood IDDM. *Diabetologia* 39:1091-1098, 1996
22. Baekkeskov S, Aanstoot H, Fu Q, Jallie M, Kim J, Quan J, Richter W, Shi Y: The glutamate decarboxylase and 38kD autoantigens in type 1 diabetes: aspects of structure and epitope recognition. *Autoimmunity* 15 (Suppl.):24-26, 1993
23. Atkinson MA, Maclaren NK: Islet cell autoantigens in insulin-dependent diabetes. *J Clin Invest* 92:1608-1616, 1993
24. Gianani R, Eisenbarth GS: Humoral autoimmunity. In *Type 1 Diabetes: Molecular, Cellular, and Clinical Immunology*. 1st ed. Eisenbarth GS, Lafferty KJ, Eds. New York, Oxford University Press, 1996, p. 209-229
25. Bonifacio E: Humoral immune markers: islet cell antibodies. In *Prediction, Prevention, and Genetic Counseling in IDDM*. Palmer JP, Ed. Chichester, U.K., Wiley, 1996, p. 43-62
26. Dotta F, Gianani R, Previti M, Lenti L, Dionisi S, D'Erme M, Eisenbarth GS, DiMario U: Autoimmunity to the GM2-1 islet ganglioside before and at the onset of type 1 diabetes. *Diabetes* 45:1193-1196, 1996
27. Daniel D, Wegmann DR: Protection of nonobese diabetic mice from diabetes by intranasal or subcutaneous administration of insulin peptide B-(9-23). *Proc Natl Acad Sci USA* 93:956-960, 1996
28. Ramiya VK, Shang X, Pharis PG, Wasserfall CH, Stabler TV, Muir AB, Schatz DA, Maclaren NK: Antigen based therapies to prevent diabetes in NOD mice. *J Autoimmun* 9:349-356, 1996
29. Atkinson MA, Bowman MA, Campbell L, Darrow BL, Kaufman DL, Maclaren NK: Cellular immunity to a determinant common to glutamate decarboxylase and coxsackie virus in insulin-dependent diabetes. *J Clin Invest* 94:2125-2129, 1994
30. Elliott JF, Qin HY, Bhatti S, Smith DK, Singh RK, Dillon T, Lauzon J, Singh B: Immunization with the larger isoform of mouse glutamic acid decarboxylase (GAD67) prevents autoimmune diabetes in NOD mice. *Diabetes* 43:1494-1499, 1994
31. Cetkovic-Cvrlje M, Gerling IC, Muir A, Atkinson MA, Elliott JF, Leiter EH: Retardation or acceleration of diabetes in NOD/Lt mice mediated by intrathymic administration of candidate β -cell antigens. *Diabetes* 46:1975-1982, 1997
32. Petersen JS, Karlens AE, Markholst H, Worsaae A, Dyrberg T, Michelsen B: Neonatal tolerization with glutamic acid decarboxylase but not with bovine serum albumin delays the onset of diabetes in NOD mice. *Diabetes* 43:1478-1484, 1994
33. Muir A, Peck A, Clare-Salzler M, Song YH, Cornelius J, Luchetta R, Krischer J, Maclaren N: Insulin immunization of nonobese diabetic mice induces a protective insulinitis characterized by diminished intraslet interferon-gamma transcription. *J Clin Invest* 95:628-634, 1995

34. Bowman MA, Campbell L, Darrow BL, Ellis TM, Suresh A, Atkinson MA: Immunological and metabolic effects of prophylactic insulin therapy in the NOD-scid/scid adoptive transfer model of IDDM. *Diabetes* 45:205-208, 1996
35. Zekzer D, Wong FS, Wen L, Altieri M, Gurlo T, von Grafenstein H, Sherwin RS: Inhibition of diabetes by an insulin-reactive CD4 T-cell clone in the nonobese diabetic mouse. *Diabetes* 46:1124-1132, 1997
36. Auger I, Toussiot F, Roudier J: Molecular mechanisms involved in the association of HLA-DR4 and rheumatoid arthritis. *Immunol Res* 16:121-126, 1997
37. Zhang ZJ, Davidson L, Eisenbarth G, Weiner HL: Suppression of diabetes in nonobese diabetic mice by oral administration of porcine insulin. *Proc Natl Acad Sci U S A* 88:10252-10256, 1991
38. Thivolet CH, Goillot E, Bedossa P, Durand A, Bonnard M, Orgiazzi J: Insulin prevents adoptive transfer of diabetes in the autoimmune non-obese diabetic mouse. *Diabetologia* 34:314-319, 1991
39. Ploix C, Bergerot I, Fabien N, Perche S, Moulin V, Thivolet C: Protection against autoimmune diabetes with oral insulin is associated with the presence of IL-4 type 2 T-cells in the pancreas and pancreatic lymph nodes. *Diabetes* 47:39-44, 1998
40. von Herrath MG, Dyrberg T, Oldstone MBA: Oral insulin treatment suppresses virus-induced antigen-specific destruction of β cells and prevents autoimmune diabetes in transgenic mice. *J Clin Invest* 98:1324-1331, 1996
41. Friedman A, Weiner HL: Induction of anergy or active suppression following oral tolerance is determined by antigen dosage. *Proc Natl Acad Sci U S A* 91:6688-6692, 1994
42. Hancock WW, Polansky M, Zhang J, Blogg N, Weiner HL: Suppression of insulinitis in non-obese diabetic (NOD) mice by oral insulin administration is associated with selective expression of interleukin-4 and -10, transforming growth factor-beta, and prostaglandin-E. *Am J Pathol* 147:1193-1199, 1995
43. Bergerot I, Ploix C, Petersen J, Moulin V, Rask C, Fabien N, Lindblad M, Mayer A, Czerkinsky C, Holmgren J, Thivolet C: A cholera toxin-insulin conjugate as an oral vaccine against spontaneous autoimmune diabetes. *Proc Natl Acad Sci U S A* 94:4610-4614, 1997
44. Weiner HL, Mackin GA, Maisui M, Orav EJ, Khoury SJ, Dawson DM, Hafler DA: Double-blind pilot trial of oral tolerization with myelin antigens in multiple sclerosis. *Science* 259:1321-1324, 1993
45. Trentham DF, Dynesius-Trentham RA, Oray EJ, Combitchi D, Lorenzo C, Sewell KL, Hafler DA, Weiner HL: Effects of oral administration of type II collagen on rheumatoid arthritis. *Science* 261:1727-1730, 1993
46. Robbins PF, Thomas JW, Jensen PE, Kapp JA: Insulin specific tolerance I. Abrogation of helper T cell activity is controlled by H-2-linked I-E genes. *J Immunol* 132:43-49, 1984
47. Kapp JA, Jensen PE, Robbins PF, Fritschle C: Insulin specific tolerance induction. II. Tolerant helper T cells can be rescued by insulin complexed to Brucella abortus. *J Immunol* 133:2888-2891, 1984
48. Hutchings P, Cooke A: Comparative study of the protective effect afforded by intravenous administration of bovine or ovine insulin to young NOD mice. *Diabetes* 44:905-910, 1995
49. Kaufman DL, Clare-Salzler M, Tan J, Forsthuber T, Ting GS, Robinson P, Atkinson MA, Sercarz EE, Tobin AJ, Lehmann PV: Spontaneous loss of T-cell tolerance to glutamic acid decarboxylase in murine insulin-dependent diabetes. *Nature* 366:69-72, 1993
50. Gottlieb PA, Handler ES, Appel MC, Greiner DL, Mordes JR, Rossini AA: Insulin treatment prevents diabetes mellitus but not thyroiditis in RT 6-depleted diabetes resistant BB/Wor rats. *Diabetologia* 34:296-300, 1991
51. Vlahos WD, Seemayer TA, Yale JF: Diabetes prevention in BB rats by inhibition of endogenous insulin secretion. *Metabolism* 40:825-829, 1991
52. Karounos DG, Bryson JS, Cohen DA: Metabolically inactive insulin analog prevents type 1 diabetes in prediabetic NOD mice. *J Clin Invest* 100:1344-1348, 1997
53. Wegmann D, Daniel D: Pathogenicity of insulin specific, Th2 T cell clones in NOD mice (Abstract). *Diabetes* 46 (Suppl. 1):40A, 1997
54. Harrison LC, Dempsey-Collier M, Kramer DR, Takahashi K: Aerosol insulin induces regulatory CD8 gamma delta T cells that prevent murine insulin-dependent diabetes. *J Exp Med* 184:2167-2174, 1996
55. Ben-Nun A, Wekerle H, Cohen IR: Vaccination against autoimmune encephalomyelitis with T lymphocyte line cells reactive against myelin basic protein. *Nature* 292:60-61, 1981
56. Beraud E, Kotake S, Caspi RR, Oddo SM, Chan CC, Gery I, Nussenblatt RB: Control of experimental autoimmune uveoretinitis by low dose T cell vaccination. *Cell Immunol* 140:112-122, 1992
57. Maron R, Zerubavel R, Friedman A, Cohen IR: T lymphocyte line specific for thyroglobulin produces or vaccinates against autoimmune thyroiditis in mice. *J Immunol* 131:2316-2322, 1983
58. Kakimoto K, Katsuki M, Hirofujii T, Iwata H, Koga T: Isolation of T cell line capable of protecting mice against collagen-induced arthritis. *J Immunol* 140:78-83, 1988
59. Elias D, Reshef T, Birk OS, Van der Zee R, Walker MD, Cohen IR: Vaccination against autoimmune mouse diabetes with a T-cell epitope of the human 65-kDa heat shock protein. *Proc Natl Acad Sci U S A* 88:3088-3091, 1991
60. Smerdon RA, Peakman M, Hussain MJ, Vergani D: Lymphocyte vaccination prevents spontaneous diabetes in the non-obese diabetic mouse. *Immunology* 80:498-501, 1993
61. Formby B, Shao T: T cell vaccination against autoimmune diabetes in nonobese diabetic mice. *Ann Clin Lab Sci* 23:137-147, 1993
62. Lider O, Reshef T, Beraud E, Ben-Nun A, Cohen IR: Anti-idiotypic network induced by T cell vaccination against experimental autoimmune encephalomyelitis. *Science* 239:181-183, 1988
63. Offner H, Jones R, Celnik B, Vandenbark AA: Lymphocyte vaccination against experimental autoimmune encephalomyelitis: evaluation of vaccination protocols. *J Neuroimmunol* 21:13-22, 1989
64. Herkel J, Brunner S, Meyer zum Buschenfelde KH, Lohse AW: Humoral mechanisms in T cell vaccination: induction and functional characterization of anti-lymphocytic autoantibodies. *J Autoimmun* 10:137-146, 1997
65. Clark L, Heber-Katz E, Rostami A: Shared T-cell receptor gene usage in experimental allergic neuritis and encephalomyelitis. *Ann Neurol* 31:587-592, 1992
66. Gregerson DS, Fling SP, Merryman CF, Zhang XM, Li XB, Heber-Katz E: Conserved T cell receptor V gene usage by uveitogenic T cells. *Clin Immunol Immunopathol* 58:154-161, 1991
67. Osman GE, Toda M, Kanagawa O, Hood LE: Characterization of the T cell receptor repertoire causing collagen arthritis in mice. *J Exp Med* 177:387-395, 1993
68. Vandenbark AA, Hashim G, Offner H: Immunization with a synthetic T-cell receptor V-region peptide protects against experimental autoimmune encephalomyelitis. *Nature* 341:541-544, 1989
69. Howell MD, Winters ST, Olee T, Powell HC, Carlo DJ, Brostoff SW: Vaccination against experimental allergic encephalomyelitis with T cell receptor peptides. *Science* 246:668-670, 1989
70. Haqqi TM, Qu XM, Anthony D, Ma J, Sy MS: Immunization with T cell receptor V beta chain peptides deletes pathogenic T cells and prevents the induction of collagen induced arthritis in mice. *J Clin Invest* 97:2849-2858, 1996
71. Kumar V, Aziz F, Sercarz E, Miller A: Regulatory T cells specific for the same framework 3 region of the Vbeta8.2 chain are involved in the control of collagen II-induced arthritis and experimental autoimmune encephalomyelitis. *J Exp Med* 185:1725-1733, 1997

72. Desquenette-Clark L, Esch TR, Otvos LJ, Heber-Katz E: T cell receptor peptide immunization leads to enhanced and chronic experimental allergic encephalomyelitis. *Proc Natl Acad Sci U S A* 88:7219-7223, 1991
73. MacNeil D, Fraga E, Singh B: Characterization of murine T cell responses to peptides of the variable region of self T cell receptor beta-chains. *J Immunol* 151:4045-4054, 1993
74. Broeren CP, Lucassen MA, van Stipdonk MJ, Van der Zee R, Boog CJ, Kusters JG, Van Eden W: CDR1 T-cell receptor beta-chain: peptide induces major histocompatibility complex class II-restricted T-T cell interactions. *Proc Natl Acad Sci U S A* 91:5997-6001, 1994
75. Kumar V, Stellrecht K, Sercarz E: Inactivation of T cell receptor peptide-specific CD4 regulatory T cells induces chronic experimental autoimmune encephalomyelitis (EAE). *J Exp Med* 184:1609-1617, 1997
76. Kuhrober A, Schimbeck R, Reimann J: Vaccination with T cell receptor peptides primes anti-receptor cytotoxic T lymphocytes (CTL) and anergizes T cells specifically recognized by these CTL. *Eur J Immunol* 24:1172-1180, 1994
77. Moreland LW, Heck LW, Koopman WJ, Saway PA, Adamson TC, Fronek Z, O'Connor RD, Morgan EE, Diveley JP, Richieri SP, Carlo DJ, Brostoff SW: V beta 17 T cell receptor peptide vaccination: in rheumatoid arthritis: results of phase 1 dose escalation study. *J Rheumatol* 23:1353-1362, 1996
78. Sarukhan A, Gombert JM, Olivi M, Bach JF, Carnaud C, Garchon HJ: Anchored polymerase chain reaction based analysis of the V beta repertoire in the non-obese diabetic (NOD) mouse. *Eur J Immunol* 24:1750-1756, 1994
79. Sarukhan A, Bedossa P, Garchon HJ, Bach JF, Carnaud C: Molecular analysis of TCR junctional variability in individual infiltrated islets of non-obese diabetic mice: evidence for the constitution of largely autonomous T cell foci within the same pancreas. *Int Immunol* 7:139-146, 1995
80. Candéas S, Katz J, Benoist C, Mathis D, Haskins K: Islet-specific T-cell clones from nonobese diabetic mice express heterogeneous T-cell receptors. *Proc Natl Acad Sci U S A* 88:6167-6170, 1991
81. Nakano N, Kikutani H, Nishimoto H, Kishimoto T: T cell receptor V gene usage of islet β cell-reactive T cells is not restricted in non-obese diabetic mice. *J Exp Med* 173:1091-1097, 1991
82. Shizuru JA, Taylor-Edwards C, Livingstone A, Fathman CG: Genetic dissection of T cell receptor V beta gene requirements for spontaneous murine diabetes. *J Exp Med* 174:633-638, 1991
83. McDuffie M: Diabetes in NOD mice does not require T lymphocytes expressing V beta 8 or V beta 5. *Diabetes* 40:1555-1559, 1991
84. Lipes MA, Rosenzweig A, Tan KN, Tanigawa G, Ladd D, Seidman JG, Eisenbarth GS: Progression to diabetes in nonobese diabetic (NOD) mice with transgenic T cell receptors. *Science* 259:1165-1169, 1993
85. Simone E, Daniel D, Schloot N, Gottlieb P, Babu S, Kawasaki E, Wegmann D, Eisenbarth GS: T cell receptor restriction of diabetogenic autoimmune NOD T cells. *Proc Natl Acad Sci U S A* 94:2518-2521, 1997
86. Conrad B, Weissmahr RN, Boni J, Arcari R, Schupbach J, Mach B: A human endogenous retroviral superantigen as candidate autoimmune gene in type 1 diabetes. *Cell* 90:303-313, 1997
87. Yamagata K, Hanafusa T, Nakajima H, Tomita K, Itoh N, Kono N, Matsuzawa Y: Restricted heterogeneity of T cell receptor V α transcripts in the pancreas of newly diagnosed insulin dependent diabetes mellitus (Abstract). *Autoimmunity* 15:46, 1993
88. Tang DC, DeVit M, Johnston SA: Genetic immunization is a simple method for eliciting an immune response. *Nature* 356:152-154, 1992
89. Ragno S, Colston MJ, Lowrie DB, Winrow VR, Blake DR, Tascon R: Protection of rats from adjuvant arthritis by immunization with naked DNA encoding for mycobacterial heat shock protein 65. *Arthritis Rheum* 40:277-283, 1997
90. Waisman A, Ruiz PJ, Hirschberg DL, Gerlman A, Oksenberg JR, Brocke S, Mor F, Cohen IR, Steinman L: Suppressive vaccination with DNA encoding a variable region gene of the T-cell receptor prevents autoimmune encephalomyelitis and activates Th2 immunity. *Nat Med* 2:899-905, 1996
91. Garza KM, Griggs ND, Tung KS: Neonatal injection of an ovarian peptide induces autoimmune ovarian disease in female mice: requirement of endogenous neonatal ovaries. *Immunity* 6:89-96, 1997
92. Türlbach M, Nouza K, Mullerova M, Pekarek J: Immunodiabetes in rabbits. *Endocrinol Exp* 22:3-18, 1988
93. Bock T, Pedersen CR, Josefsen K, Bottazzo GF, Palmer JP, Buschard K: No risk of diabetes after insulin-shock treatment. *Lancet* 339:1504-1508, 1992
94. Keller RJ, Eisenbarth GS, Jackson RA: Insulin prophylaxis in individuals at high risk of type 1 diabetes. *Lancet* 341:927-928, 1993
95. Heath WR, Miller JFAD: Oral tolerance: feeding autoantigens can exacerbate rather than ameliorate autoimmune disease. *J NIH Res* 9:35-39, 1997
96. Blanas E, Carbone FR, Allison J, Miller JF, Heath WR: Induction of autoimmune diabetes by oral administration of autoantigen. *Science* 274:1707-1709, 1996
97. Genain CP, Abel K, Belmar N, Villinger E, Rosenberg DP, Linington C, Raine CS, Hauser SL: Late complications of immune deviation therapy in a nonhuman primate. *Science* 274:2054-2057, 1996
98. Wilson DB, Golding AB, Smith RA, Dafashy T, Nelson J, Smith L, Carlo DJ, Brostoff SW, Gold DP: Results of a phase I clinical trial of a T-cell receptor peptide vaccine in patients with multiple sclerosis. I. Analysis of T-cell receptor utilization in CSF cell populations. *J Neuroimmunol* 76:15-28, 1997
99. Mor G, Singla MG, Steinberg AD, Hoffman SL, Okuda K, Klinman DM: Do DNA vaccines induce autoimmune disease? *Hum Gene Ther* 8:293-300, 1997
100. Ziegler AG, Schwertner R, Rahl W, Bachmann W, Standl E: Schwabing insulin prophylaxis (SIP) in relatives at high risk for type 1 diabetes. In *Excerpta Medica*. Baba S, Kaneko T, Eds. New York: Elsevier, 1995, p. 1080-1082
101. Yu L, Rewers M, Gianani R, Kawasaki E, Zhang Y, Verge C, Chase P, Klingensmith G, Erlich H, Norris J, Eisenbarth GS: Anti-islet autoantibodies develop sequentially rather than simultaneously. *J Clin Endocrinol Metab* 81:4264-4267, 1996
102. Rewers M, Bugawan TL, Norris JM, Blair A, Beaty B, Hoffman M, McDuffie RS Jr, Hamman RF, Klingensmith G, Eisenbarth GS, Erlich HA: Newborn screening for HLA markers associated with IDDM: Diabetes Autoimmunity Study in the Young (DAISY). *Diabetologia* 39:807-812, 1996